whereas we assumed the outer dielectric constant to be $D_0 = 78$.[7,9] The latter (zero-order) approximation seems to be appropriate for those ion pairings depicted in Figure 1, where 22 other water molecules are present in the neighborhood.[10a,c] Then, the effective dielectric constant, D_{eff}, is given by the Westheimer-Kirkwood equation[7a] (eq 2) and the intermolecular electrostatic energies

$$\frac{1}{D_{eff}} = \frac{1}{D_i} + \left(\frac{1}{D_o} - \frac{1}{D_i}\right)\sum_{n=0}^{\infty} \frac{U_n}{[1 + (D_i/D_o)]C_n} \qquad (2)$$

calculated by using D_{eff} ($r_{ij} < 15$ Å, $|q| > 0.01$, eq 1, $D = D_{eff}$) are shown in the last column of Table I. To each ionic residue a given counterion, H_3O^+ or OH^- with point charge on the oxygen atom, was assigned in same place as the nearest water molecule.[10b]

Most of the repulsive interactions between (partial) charges of the same sign (espe Lys60E···Arg17I, 20I, Lys46I) were markedly reduced, e.g., from E_{el} ($D = 2$) = +14.80 kcal mol^{-1} to E_{el} (D_{eff}) = +0.416 kcal mol^{-1} for NH_3^+ (Lys60E)···guanidinium$^+$ (Arg20I) interaction, as expected by taking proximal water molecules into account (Table I).[11a] Thus, the overall electrostatic interaction without bridges became attractive ($E_{el}^{nonbridge}$ = −38.7 kcal mol^{-1})[11b] (Table I).

The electrostatic energy due to water (counterion) bridging (E_{el}^{bridge}) which is the sum of the following two interactions—counterions of BPT···BPTI, where m is the number of counterions of BPT (up to 13) and n is the number of partial point charges on BPTI under consideration (up to 855) and BPT···counterions of BPTI, where m is the number of partial point charges on BPT under consideration (up to 3224) and n is the number of counterions of BPTI (up to 6)—was calculated by using eq 1 ($D = D_{eff}$). Table II gives the calculated E_{el}^{bridge} values obtained for the counterions W1, W2, and W4 when W3 and W5 are neutral water particles.[12] The overall E_{el}^{bridge} value summed over all (partial) point charge pairs and $E_{el}^{nonbridge}$ are given in Table III together with a correction term, E_{el}^{water}, the electrostatic energy due to interactions between counterions of E and I.

It is evident that W1, W2, and W4 contribute significantly to the stabilization of the EI complex by behaving as OH^- (counteranion), as exemplified by the negative E_{el}^{bridge} values of −20.9, −15.0, and −7.3 kcal mol^{-1} for the interactions OH^-(W1)···BPTI, BPT···OH^-(W2), BPT···OH^-(W4), respectively (Table II).

Most importantly, the overall electrostatic energy due to the water (counterion) bridging was found to be −31.6 kcal mol^{-1} (Table III). Evidently, overall E_{el}^{bridge} is one of the most outstanding terms contributing to the electrostatic interaction (E_{el}^{total} = −62.4 kcal mol^{-1}) in the EI complex (Table III).

In conclusion, the water bridged cation-cation interaction significantly stabilizes the BPT-BPTI complex, and also any repulsive electrostatic interaction is markedly reduced by water molecules in the proximity. These may be seen in protein-protein interactions in general.

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(10) (a) The total number of water molecules (except W1-W5), which was found within 5-Å distance from any of 10 ionic residues (Figure 1) but not placed between E and I, was 22. (b) Within 5 Å from ionic residue. (c) Lys46I and Arg20I find very few number of atoms on the residue of E in their neighborhood, viz., W1-W4 are very close to the complex surface.

(11) (a) The result of significant reduction of repulsive interaction was unchanged, even when $D_0 = 70$ was assumed, as exemplified by E_{el} ($D = D_{eff}$) = −0.459 kcal mol^{-1} for NH_3^+(Lys60E)···guanidinium$^+$(Arg20I) interaction or overall $E_{el}^{nonbridge}$ ($D = D_{eff}$) = −39.6 kcal mol^{-1}. (b) Most of this remarkable EI complex stabilization comes from interactions of (intermolecular) ion pairs separated by <10 Å, since the $E_{el}^{nonbridge}$ value amounted to −35.9 kcal mol^{-1} when $r < 10$ Å was assumed.

(12) This counterion configuration was the optimized one which led to the largest calculated E_{el}^{bridge} value for W1-W5, by varying the kind, +, neutral, or −, of counterions on those water positions.

Optically Detected Magnetic Resonance Evidence for Carcinogen-Nucleic Acid Interaction in the Tetrahydro-9,10-epoxybenzo[*e*]pyrene-DNA Adduct

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There is now strong evidence that the carcinogenic action of polycyclic aromatic hydrocarbons (PAH) is mediated via metabolically activated intermediates[1] and that the critical initiating step in carcinogenesis is the covalent binding of these intermediates to DNA.[2,3] Recent results have shown that the qualitative nature of the PAH-DNA binding site may be correlated with its carcinogenicity,[4] indicating that structural characterization of carcinogen-nucleic acid adducts may prove important in relating these complexes to subsequent events in carcinogenesis.

Here we use the technique of optically detected magnetic resonance (ODMR)[5] to probe the structure of the adduct of the tetrahydro-9,10-epoxy derivative of benzo[*e*]pyrene (BePE, Figure 1a)[6] with DNA. BePE, while not an in vivo metabolite of benzo[*e*]pyrene,[7,8] is a potent mutagen and tumorigen[7,9] and is assumed to initiate carcinogenesis by its direct binding to DNA.[7,9] Preliminary linear electric dichroism results have indicated that the pyrene chromophore in the BePE-DNA adduct is approximately parallel to the planes of the DNA bases,[10] raising the possibility that the chromophore may be intercalated in the nucleic acid. However, these results alone are only suggestive of intercalation and do not exclude a completely nonintercalated adduct in a base parallel conformation. In the present study, we obtain ODMR results indicating that the pyrene triplet chromophore in the BePE-DNA adduct exists in a heterogeneous environment intermediate between that expected for intercalation and complete solvent exposure.

The BePE-DNA in vitro adduct was prepared analogously to the benzo[*a*]pyrene adduct,[11] yielding ca. 2 adducts per 1000 nucleotides. All studies on the adduct were done in 1:1 (v/v) ethylene glycol–sodium cacodylate buffer. The epoxide was acid hydrolyzed into the corresponding H_4-9,10 diol (BePD) which was used to model environmental effects on the BePE-DNA adduct, as both possess nearly identically substituted chromphores and the BePD is conveniently stable and soluble in a variety of solvents.

We chose the two solvent environments for the BePD of dry pyridine and ethylene glycol-buffer, so as to model the extreme possibilities of complete adduct chromophore intercalation between nucleic acid bases and total solvent exposure, respectively. All glycol-buffer solutions were quickly immersed in liquid nitrogen

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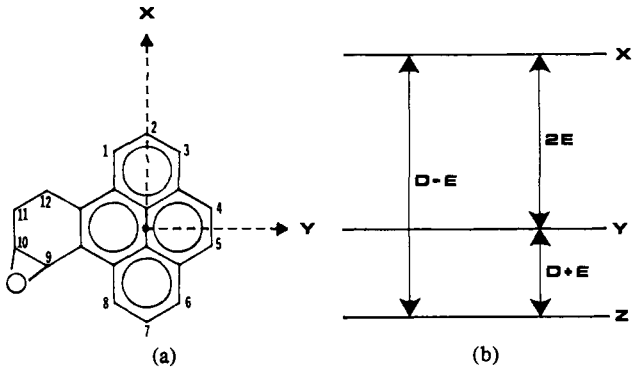

Figure 1. (a) Tetrahydro-9,10-epoxy derivative of benzo[*e*]pyrene with principal zero-field axis system[21] (*z* axis into plane of paper). (b) Sublevel energy splitting diagram[21] with ODMR transitions.

modeled by the pyridine spectrum, and free solvent exposure, represented by the glycol–buffer peak. The adduct transition is broadened over that of the diol in the same solvent and has two reproducible shoulders nearly corresponding to diol maxima in pyridine and glycol–buffer, indicating that considerable heterogeneity may exist in the BePE binding site, ranging from almost complete solvent exposure to considerable base-like interaction. The 2E spectra show a similar shift and broadening in the DNA adduct.

We determined the individual sublevel total decay rates (k_x, k_y, and k_z) in the diol and the covalent adduct, as these are expected to be sensitive to the chromophore's environment. The rates were obtained by microwave-induced delayed phosphorescence[15] and fast passage[16] methods. For the BePE–DNA complex these measurements yielded (in s^{-1}) $k_x = 1.0 \pm 0.1$, $k_y = 2.6 \pm 0.2$, and $k_z = 0.5 \pm 0.1$, while the diol in glycol–buffer gave $k_x = 1.2 \pm 0.1$, $k_y = 2.9 \pm 0.2$, and $k_z = 0.6 \pm 0.1$. The averages of these values, $(k_x + k_y + k_z)/3$, agree with the 77-K phosphorescence decay rates of 1.4 ± 0.1 and 1.5 ± 0.1 for the adduct and diol, respectively. Thus there are no significant differences in the measured rates for the free and bound chromophores and no anomalous increase in k_z upon binding, which has been noted for several PAH's physically intercalated in DNA.[17,18]

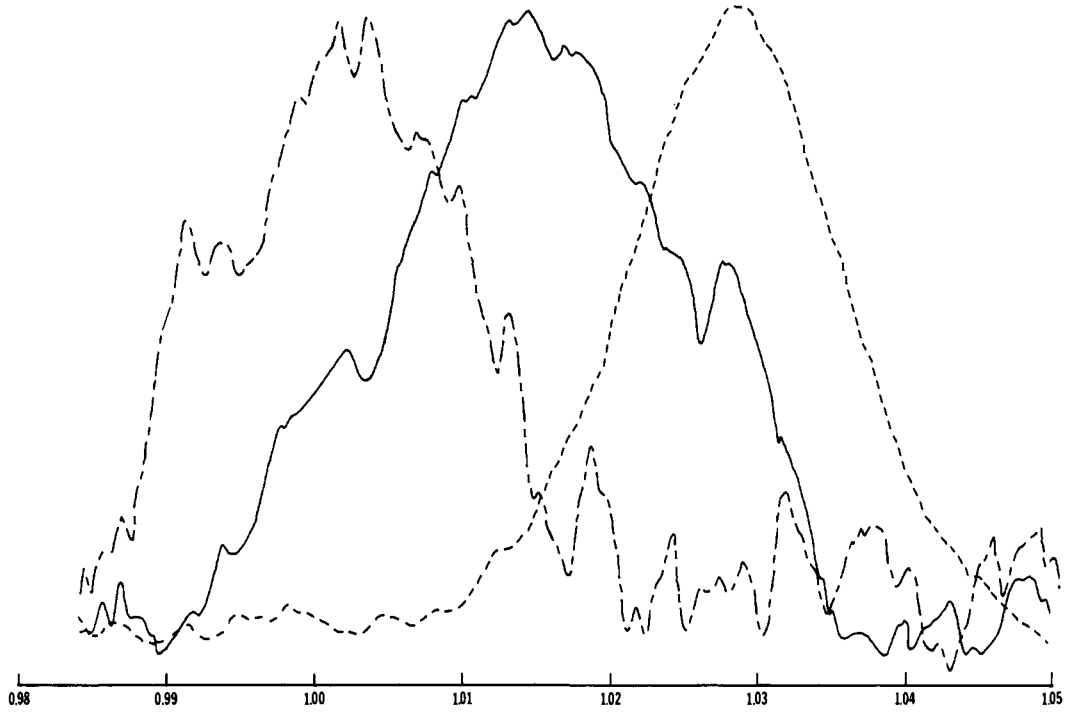

Figure 2. ODMR of D + E transition in BePD in pyridine (---), and glycol buffer (---), and BePE–DNA adduct in glycol buffer (—).

to form glasses, but the pyridine samples were prepared by a modified cryogenic Bridgman technique.[12]

The 77-K phosphorescence spectra of the BePE–DNA adduct, the BePD in pyridine, and the BePD in glycol–buffer all showed nearly identical pyrene-like phosphorescence,[13] with peaks at 581 nm. The 2-K ODMR spectra[14] (detected at 581 nm in all cases) of the D + E transition (Figure 1b) yielded maxima at 1.015 ± 2 GHz for the DNA adduct and 1.031 ± 2 and 1.003 ± 2 GHz for the diol in glycol–buffer and pyridine, respectively. The 2E (Figure 1b) transitions peaked at 2.091 ± 2 GHz for the adduct and 2.108 ± 2 GHz for the diol in glycol buffer. No 2E signal could be obtained in pyridine, probably due to fast relaxation between the *y* and *z* sublevels. The D–E transitions were not observed in any of the systems.

Figure 2 shows that the D + E transition in the DNA adduct is intermediate in frequency between the diol in pyridine and in glycol–buffer, implying that the adduct chromophore's degree of interaction with the nucleic acid is between that of intercalation,

It is worthwhile to point out that in our earlier work on the benzo[*a*]pyrenediol epoxide–DNA (BaPDE) system,[14] which also contains the pyrene chromophore, we found no significant shifts in either the D + E or the 2E transitions, when the BaPDE adduct was compared to the hydrolyzed tetraol, and noted no increase in k_z. These results, in contrast to those presented here, emphasized the lack of chromophore–DNA interaction, and pointed to an essentially solvent exposed conformation, in agreement with previous fluorescence quenching[11] and linear dichroism[19] results.

In summary, we have presented ODMR results indicating that

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the pyrene chromophore in the DNA adduct of BePE exists in an environment somewhere between complete intercalation and solvent exposure. Whether this intermediate and heterogeneous environment results from different carcinogen binding sites on an intact nucleic acid structure or is a consequence of variations in local DNA denaturation[20] is a question we will address in future experiments. In any case, it is clear that for these systems, ODMR reveals a substantial amount of detail about carcinogen-DNA interactions not present in conventional phosphorescence results.

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Stereoselective Synthesis of Steroid Side Chain: A Route to De-*AB*-cholestan-9-one

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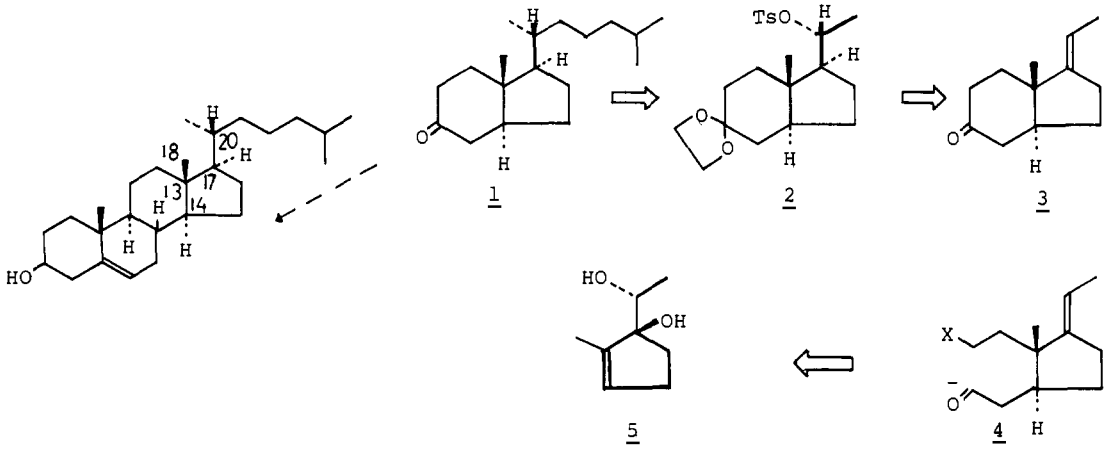
Highly regio- and stereoselective construction of steroid side chains is a current problem[1] in the synthesis of various physiologically active steroids and metabolites of vitamin D. The most crucial problem inherent in the synthesis of steroid side chains is the introduction of asymmetric centers at C(17) and C(20) (steroidal numbering). For this purpose, the Carroll[2] or oxy-Cope[3] rearrangement at the steroidal allylic alcohol moiety and nucleophilic attack at π-allylpalladium intermediates[4] derived from steroidal olefins were previously used as key stereodirecting processes. The conformational rigidity of [2.2.1]heptane derivatives[5] was also quite useful. We describe here the successful construction of the hydrindanone **3** and its stereocontrolled conversion to de-*AB*-cholestan-9-one (**1**).

In our synthetic plan (Scheme I), the key step is the stereospecific displacement (S_N2) of the secondary tosylate **2**, derived from the [17(20)*E*]-olefin of **3**, with the carbanion of **16** to produce the right stereochemistry at C(20). The stereocontrolled construction of **3** involves two Claisen rearrangements of **5**, the first one to introduce the acyl chain at C(14) and the second to introduce the chain at C(13) with the right trans stereochemistry between C(13) methyl (18-methyl) and C(14) hydrogen, as well as the geometry of the [17(20)*E*]-olefin, and subsequent efficient cyclization via acyl carbanion **4**.

Thus the allyl alcohol **5** was our initial synthetic target and easily prepared from 2-methylcyclopentenone (**6**) in the following way (Scheme II). Addition of the enone **6** (50 mmol), at -78 °C under nitrogen, to a solution of (α-ethoxyvinyl)lithium,[6] prepared from ethyl vinyl ether (90 mmol) and *tert*-butyllithium (75 mmol) in dry THF at 0 °C, and the hydrolysis of the resulting vinyl ether with aqueous acid (0.1 N HCl/THF, 10 min at 0 °C) gave the ketone **7** in 70% overall yield.[7] The highly stereoselective reduction of the ketone **7** with sodium borohydride in THF/H_2O at 0 °C gave the diol **5**[7] (R_f = 0.27, 4:1 ether-*n*-hexane) in 80% yield, and its isomer (R_f = 0.20) was formed in 8% yield. They were easily separated by chromatography on silica gel (elution with 25% ether in *n*-hexane). The selective acetylation of the secondary alcohol in the diol **5** with acetyl chloride in pyridine at room temperature gave the acetate **8** in 71% yield. The Johnson Claisen rearrangement [$CH_3C(OEt)_3$, propionic acid, at 120 °C for 3 h] of the allyl alcohol **8** gave the ester **9a** in 57% yield. The hydrolysis of the acetate **9a** in methanolic K_2CO_3 at 0 °C for 3 h gave the ester **9b** in 70% yield: NMR (CCl_4) δ 1.23 (3 H, d, J = 6 Hz, CH_3), 1.63 (3 H, br s, C=CCH_3), 3.67 (3 H, s, $OCOCH_3$), 4.67 (2 H, q, J = 6 Hz, C*H*(OH)CH_3); IR (neat) 3400 and 1735 cm^{-1}.

Then we attempted to establish the right stereochemistry between C(13) and C(14) by the second Claisen rearrangement of the vinyl ether of the allyl alcohol **9b** based on the consideration of two possible Claisen chair-like transition states **10a** and **10b** (Scheme III). In **10b** clearly there are greater steric interactions than in **10a**. Consequently, the Claisen rearrangement should proceed via the transition state **10a** which gives the trans stereochemistry at C(13) methyl and C(14) hydrogen as well as the

Scheme I



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